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Colchicine induced chromosome mosaicism in chili pepper (*Capsicum annuum* L.)

K G RAJA RAO, I HARINI* and O ANIEL KUMAR
Department of Botany, Andhra University, Waltair 530 003, India
*Department of Botany, Nagarjuna University, Nagarjunanagar 522 510, India

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Abstract. One of the colchicine treated plants of the cultivar Co_2 of *Capsicum annuum* L. was found to be a chromosome mosaic without seed-set and with as low as 4·95% pollen fertility. The growth of the plant was stunted. Its chromosome number (2n) varied from 38 to 96. Chromosome associations and chiasma frequencies were studied in each of the chromosome classes. Bivalents and association of 4 chromosomes were prevalent. Univalents were common. Laggards were observed at anaphases I and II. The chromosome mosaicism is attributed to the effect of colchicine on the spindle and physiological process.

Keywords. *Capsicum annuum*; colchicine; chromosome mosaicism; aneusomaty; chili pepper.

1. Introduction

The occurrence of different chromosome numbers in meiocytes of the same anther or an organism is termed chromosome mosaicism or aneusomaty. This phenomenon is encountered occasionally in anther or root tip cells (Guildenhuys and Brix 1958; Fukumoto 1962). This anomaly may occur either spontaneously or induced through chemicals or due to environmental factors. Chromosome mosaicism has been recorded in natural polyploids (Thompson 1962; Pantulu and Narasimha Rao 1977; Latha Kumari and Jayalakshmi 1984; Rao and Nirmala 1986), inter-specific and interracial hybrids (Shahare and Shastry 1963; Yang 1965; Venkateswarlu and Raja Rao 1979), in synthetic auto- and amphiploids (Sachs 1952; Yang 1964; Venkateswarlu and Krishna Rao 1969; Lydia Prasad 1982), and in mutants induced through chemical mutagens (Vaarama 1949; Sharma and Bhattacharjee 1953; Mitra and Steward 1961; Ross 1962; Rajhathy 1963; Siddiq 1967; Gottschalk 1971; Kasha 1974; Sadasivaiah and Lesins 1974; Rao and Rao 1977). Inspite of its wide occurrence, the mechanism involved and the exact causes are poorly understood. The present paper documents the cytomorphology of colchicine induced chromosome mosaicism in cultivar Co_2 of chili pepper.

2. Materials and methods

The apical actively growing vegetative buds of 10 days old seedlings of the cultivar Co_2 of *Capsicum annuum* L. were treated for 18 h with 0·3% aqueous solution of colchicine.

For cytological analysis, young flower buds were fixed for 24 h in 1:3 acetic acid and absolute alcohol mixture and then transferred to 70% alcohol.

3. Observations

Morphologically the chromosome mosaic plant showed stunted growth, fewer branches, leaves thick and dark green in colour and abnormal in shape. Further, it is characterised by relatively larger flowers, irregular flower openings, compared to the sib tetraploid and diploid progenitor (figure 1).

A total of 280 PMCs were analysed at diakinesis and metaphase I. The chromosome number (2n) varied from 38 to 96 (figure 3) and different chromosome classes were discernible. Not even a single pollen *mether* cell exhibited the normal chromosome number (24) of the species. Though the somatic chromosome numbers of 48 and 96 were recorded in higher frequencies (figures 4 and 6) than others, no single chromosome number could be judged as distinctly predominant (table 1). All cells undergo meiosis synchronously. In the anther, all the cells were found to be at the same stage of meiosis and further the cells of different chromosome number were found to be intermixed. In general, the size of the cells was apparently bigger when more than 48 chromosomes are present (figure 5). However, exceptionally a few cells were large eventhough they have fewer chromosomes. The average chromosome number per cell was 46·41. Chromosome fragments varying from 1 to 3 per PMC were also recorded in 0·2% of the cells. Variation in the number of chromosomes and fragments were also found in tapetal cells (figure 2).

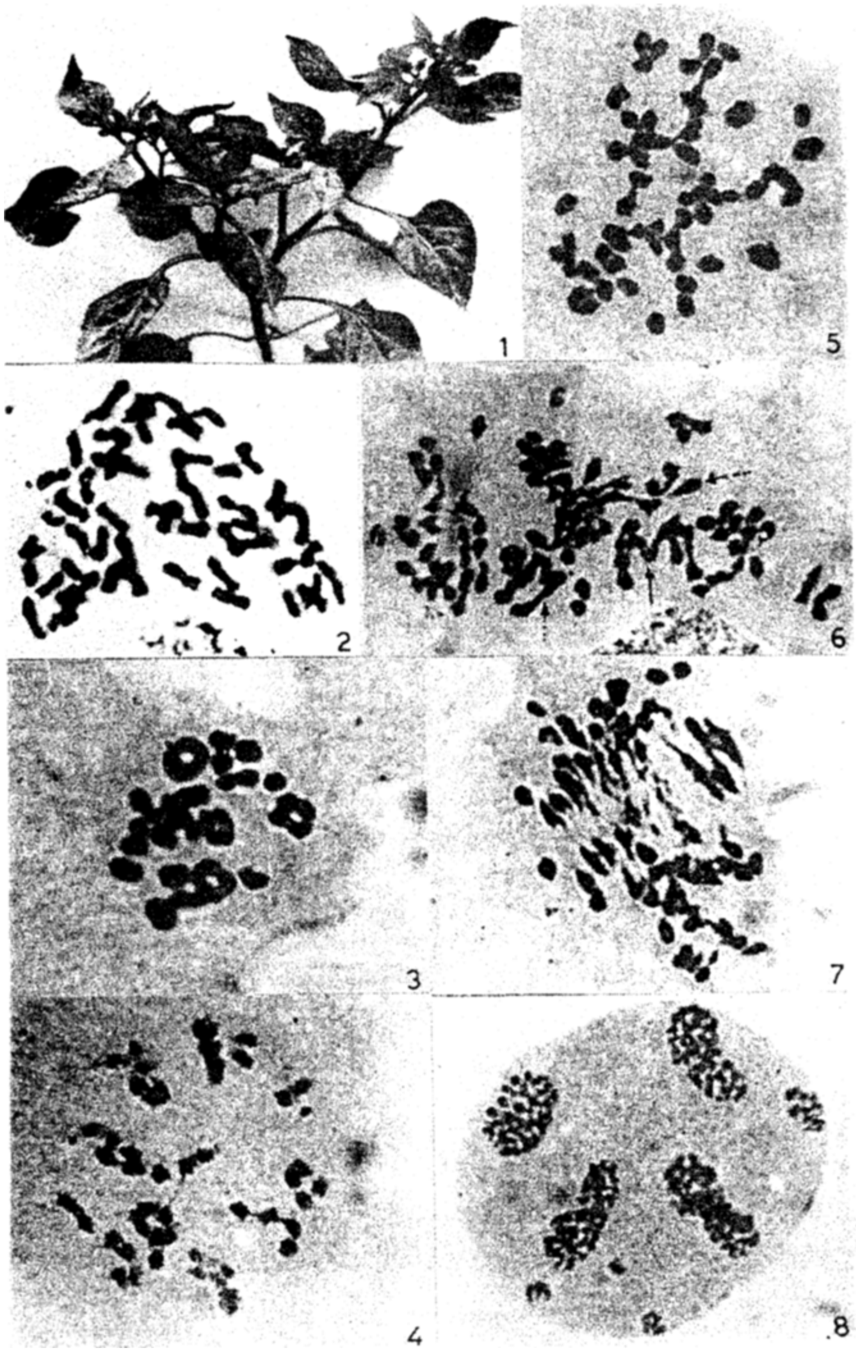
Chromosome associations and chiasma frequency were studied from each of the chromosome classes (table 1). Chromosome associations, higher than bivalents, involving upto 8 chromosomes, were frequent; however, those involving 5 or more chromosomes were not more than two per cell. All the associations were of chain-type. The mean frequencies of higher associations are depicted in table 2. Bivalents occurred in a greater frequency than all other associations and among them rod bivalents occurred in a greater frequency. The mean frequency of univalents per cell varied from 4·37 to 12·91. The mean number of half chiasmata per nucleus in various chromosome classes was different (table 1). In some of the chromosome classes the number of chromosomes involved in higher associations were always more than the number of homologous chromosomes possibly available for pairing as multivalents. Further the chromosomes involved in such associations were not of the same size, some of the chromosomes in such associations were as small as fragments.

At anaphase I, apart from irregular chromosome disjunction, laggards ranging from 1 to 6 and a single bridge were encountered in 158 (79%) of 200 cells examined (figure 7). The chromosome number at this stage in some PMCs could not be counted with certainty because of heavy clumping at the poles and considerable overlapping of the laggards and fragments. These irregularities were passed on to the second meiotic divisions as well (figure 8). Though meiotic irregularities occurred in a greater frequency, fewer polyads were formed at the tetrad stage. Pollen grains of varying sizes were observed. The pollen fertility was very low (4·95%) in the chromosome mosaic plant while it was high in the sibtetraploids (56·75%) and the diploid progenitor (93·25%).

The inheritance pattern of mosaicism could not be established since no seed was obtained on open pollination, selfing or crossing with 4n and 2n plants.

4. Discussion

The present investigation reveals that variation in chromosome number within the



Figures 1–8. Morphology and cytology of colchicine induced chromosome mosaic plant of chili pepper. **1.** Twig of chromosome mosaic plant. **2.** Tapetal cell showing 38 Chromosomes (× 1800). **3.** Metaphase I, 4 IV + 13 II + 2 I (2n = 44) (× 1800). **4.** Diakinesis, 4 IV + 2 III + 12 II + 2 I (2n = 48) (× 1800). **5.** Diakinesis, 4 IV + 2 III + 18 II + 8 I (2n = 66) (× 1800). **6.** Metaphase I, 1 VIII (↑) + 1 VI (↑) + 1 V (↑) + 7 IV + 4 III + 16 II + 5 I (2n = 96) (× 1800). **7.** Anaphase I showing bridges and lagging chromosomes (× 1800). **8.** Telophase II showing more than four groups (× 1800).

Table 1. Intraplant chromosome numerical variation and meiotic pairing in the different chromosome classes in *C. annuum* L.

Chromosome class (2n =)	Chromosome associations								Chiasmata	Half chiasmata per chromosome	No. of cells	Per cent
	VIII	VII	VI	V	IV	III	II	I				
38	—	—	—	—	4·45 ± 0·35	1·2 ± 0·13	5·5 ± 0·47	4·37 ± 0·47	27·5 ± 0·56	0·72	24	8·57
40	—	—	—	—	1·43 ± 0·38	—	14·9 ± 0·76	4·37 ± 0·36	27·5 ± 0·24	0·68	16	5·71
44	—	—	—	—	5·77 ± 0·09	—	7·4 ± 0·32	6·0 ± 0·66	30·66 ± 0·88	0·69	18	6·42
48	—	—	—	—	3·96 ± 0·25	1·84 ± 0·19	10·87 ± 0·57	4·84 ± 0·25	33·12 ± 0·49	0·69	32	11·42
50	—	—	—	—	3·2 ± 0·29	3·0 ± 0·24	11·23 ± 0·45	5·6 ± 0·63	33·53 ± 0·38	0·67	13	4·64
52	—	—	—	—	3·88 ± 0·39	2·8 ± 0·18	11·88 ± 0·63	4·0 ± 0·64	30·77 ± 0·52	0·59	9	3·21
58	—	—	—	—	4·4 ± 0·26	2·8 ± 0·21	13·06 ± 0·41	5·86 ± 0·75	36·86 ± 1·01	0·63	14	5·00
60	—	—	—	—	4·71 ± 0·23	2·85 ± 0·24	12·42 ± 0·48	7·85 ± 0·57	42·21 ± 1·00	0·70	15	5·35
62	—	—	—	—	2·18 ± 0·27	2·41 ± 0·18	19·12 ± 0·81	7·7 ± 0·34	36·23 ± 0·89	0·58	17	6·07
66	—	—	—	—	4·31 ± 0·23	2·54 ± 0·10	17·61 ± 0·54	5·69 ± 0·43	43·5 ± 0·55	0·65	26	9·28
70	0·58 ± 0·14	—	1·42 ± 0·14	—	3·75 ± 0·13	2·83 ± 0·23	13·92 ± 0·52	5·5 ± 0·38	57·17 ± 1·26	0·81	12	4·28
72	0·4 ± 0·15	—	0·6 ± 0·21	—	3·5 ± 0·35	4·0 ± 0·24	16·8 ± 1·18	4·8 ± 0·39	46·4 ± 1·12	0·64	10	3·57
84	1·0 ± 0·10	—	1·27 ± 0·13	—	4·9 ± 0·27	2·55 ± 0·23	14·09 ± 0·27	12·91 ± 0·37	55·73 ± 0·83	0·66	11	3·92
90	0·33 ± 0·19	—	0·33 ± 0·19	—	9·33 ± 0·96	5·67 ± 0·77	11·33 ± 1·35	6·67 ± 0·19	65·0 ± 1·73	0·72	6	2·14
96	0·51 ± 0·07	—	1·18 ± 0·05	0·26 ± 0·07	6·39 ± 0·23	3·30 ± 0·13	19·42 ± 0·34	8·93 ± 0·49	67·79 ± 0·54	0·70	57	20·35
										No. of PMCs	280	

Table 2. Frequencies of higher chromosome associations in the chromosome mosaic plant of chili pepper (*C. annuum* L.).

Higher chromosome associations	Total number	Maximum found in a cell	Number of cells	Mean number per cell
VIII	53	1	53	0·19 ± 0·03
VI	106	2	87	0·37 ± 0·03
V	17	2	15	0·06 ± 0·02
IV	1275	11	273	4·50 ± 0·13
III	666	7	239	2·30 ± 0·08

same anther is random rather than segregation into multiples of basic number (X = 12). According to Levan (1941) the origin of higher ploid cells along with haploid PMCs in *Phleum pratense* is due to the fusion of neighbouring cells as early as at preleptotene stage and he termed this as 'syncyteformation'. The presence of various sets of chromosomes in the barley mutant was described by Smith (1942) as multiploid sporocyte which was found to be a monogenic recessive. He opined that this was apparently accomplished by assembling chromosomes from different cells rather than by repeated divisions of chromosomes within the individual cells. Sachs (1952) suggested that chromosome mosaicism probably arose by gene controlled spindle abnormalities in the premeiotic mitosis. However, others suggested this to the formation of separate and independent spindles in a cell (Vaarama 1949; Britton and Hull 1957) or a type of segregational mitosis (Menzel and Brown 1952). Vaarama (1949), Franzke and Ross (1952), Siddiq (1967), Rao and Rao (1977) and Lydia Prasad (1982) reported that chromosome mosaicism could be induced by colchicine. It has been reported that ribonucleo proteins (Kaufman and Das 1955), sodium nucleate solution (Huskins 1948, 1949) and antibiotics (Wilson 1950) when applied to the somatic tissues produced meiotic abnormalities including chromosome mosaicism. Shahare and Shastry (1963) encountered chromosome mosaics in cultivated roses and further they attributed it to chromosome elimination. Chennaveeraiah and Wagley (1985) have suggested endoreduplication as another possible reason for the occurrence of mosaicism in garden crotons. Recently, Panda (1985) opined that mosaicism encountered in a different cultivar of chili pepper was genotypically controlled. The abnormalities may have originated in the premeiotic mitosis since chromosome number variation was also observed in the tapetal cells. Nirmala and Rao (1984) also observed variation of chromosome number in the tapetal cells of *Coix gigantia*. Further, chromosome number variation within the same anther or an organism may bring about pollen polymorphism and the same has been observed in the present investigation. Lavania (1982) has also reported pollen polymorphism in the mosaic plant of *Lathyrus sativus*.

In the present study all the chromosomes that were involved in higher associations such as VIII or VI and V were not of the same size thereby indicating that the higher associations could be due to reciprocal interchanges.

In an earlier study on polyploidization of *C. annuum* it was suggested that the optimum ploidy level for chili pepper is tetraploidy and beyond this level it failed to survive (Panda *et al* 1984). In view of this probably most of the hypho and hyper ploid PMCs gave rise to abortive gametes. This view point gains support from the very low pollen fertility and lack of seed set observed in the mosaic plant of the present investigation.

Finally, the chromosome mosaicism encountered in the present study may be attributed to the effect of chemical colchicine which may have affected both the spindle and the normal physiological process in the plant causing cellular effects. In the light of all these events, the mosaic plant was found to be highly unstable and did not transplant seed as expected. Therefore the genetic basis for this character (mosaicism) could not be established.

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References

Britton D M and Hull J L 1957 Meiotic instability in *Rubus*; *J. Hered.* **48** 11–20

Chennaveeraiah M S and Wagley S K 1985 Chromosome mosaicism in cultivars of garden crotons (*Codiaeum variegatum* Blume); *Nucleus* **28** 8–13

Franzke C J and Ross J G 1952 Colchicine induced variants in *Sorghum*; *J. Hered.* **43** 106–115

Fukumoto M 1962 Nuclear instability and chromosomal mosaicism in high ploids of *Solanum* species and hybrids; *Jap. J. Bot.* **18** 19–53

Gottschalk W 1971 The phenomenon of asymmetric genomic reduction; *J. Indian Bot. Soc. Golden Jubilee* **A50** 308–317

Guildenhuys P and Brix K 1958 Cytological abnormalities in *P. durum*; *Heredity* **12** 441–452

Huskins C L 1948 Chromosome multiplication and reduction in somatic tissue; *Nature (London)* **161** 80–83

Huskins C L 1949 The nucleus in development and differentiation and the experimental induction of Meiosis; *Proc. 8th Int. Genetics Congr. Hereditas (Suppl)* pp 274–285

Kasha K J (ed) 1974 Haploids from somatic cells; in *Haploids in higher plants* (Canada: University of Guelph) pp 67–87

Kaufman B P and Das N K 1955 The role of ribonucleoproteins in the production of mitotic abnormalities: *Chromosoma* 7 19–38

Latha Kumari A and Jayalakshmi K 1984 Effects of B-Chromosomes on A Chromosome Chiasma distribution in a sectorial tetraploid pearl millet plant from a West African Cultivar; *Curr. Sci.* **53** 657–658

Lavania U C 1982 Chromosomal instability in *Lathyrus sativus* L.; *Theor. Appl. Genet.* **62** 135–138

Levan A 1941 Syncyte formation in the pollen mother cells of haploid *Phleum pratense*; *Hereditas* **27** 243–252

Lydia Prasad Y 1982 *Cytogenetical and Palynological studies in the genus Physalis* L., Ph.D. thesis, Andhra University, Waltair

Menzel M Y and Brown M S 1952 Polygenomic hybrids in *Gossypium* II. Mosaic formation and somatic reduction; *Am. J. Bot.* **39** 56–69

Mitra J and Steward F C 1961 Growth induction cultures of *Haplopappus gracilis* II. The behaviour of the nucleus; *Am. J. Bot.* **48** 358–368

Nirmala A and Rao P N 1984 Chromosome instability in *Coix gigantia* Koen (Maydeae); *Can. J. Genet. Cytol.* **26** 334–338

Panda R C 1985 *Cytogenetic studies in Chili pepper* (*Capsicum L*), Ph.D. thesis, Andhra University, Waltair

Panda R C, Aniel Kumar O and Raja Rao K G 1984 Cytomorphology of induced octoploid chili pepper (*Capsicum annuum* L.); *Theor. Appl. Genet.* **68** 567–570

Pantulu J V and Narasimha Rao G J 1977 Genetically controlled chromosome numerical mosaicism in pearl millet; *Proc. Indian Acad. Sci.* **B86** 15–22

Rajhathy T 1963 Chromosome mosaics and the recovery of the original strain from octoploid *Hordeum murinum*: *Vererbungsl* **94** 269–279

Rao P N and Nirmala A 1986 Chromosome numerical mosaicism in pearl millet (*Pennisetum americanum* (L.) Leeke) *Can. J. Genet. Cytol.* **28** 203–206

Rao P N and Rao R N 1977 Colchicine induced intraplant chromosome variation in tomato; *J. Cytol. Genet.* **12** 26–29

Ross J G 1962 Proof of somatic reduction after Colchicine treatment using marked chromosomes; *Manit. Med. Rev.* **42** 536–539

Sachs L 1952 Chromosome mosaics in experimental amphidiploids in the Triticinae; *Heredity* **6** 157–170

Sadasivaiah R S and Lesins K 1974 Reduction of chromosome number in root tip cells of *Medicago*; *Can. J. Genet. Cytol.* **16** 219–227

Shahare M L and Shastry S V S 1963 Meiosis in garden roses; *Chromosoma* **13** 702–724

Sharma A K and Bhattacharjee D 1953 Somatic reduction in untreated leguminous plants; *Genetica (The Hague)* **26** 410–414

Siddiq E A 1967 Colchicine induced chromosome mosaic in *Sorghum vulgare* L.; *Proc. Indian Acad. Sci.* **B65** 275–279

Smith L 1942 Cytogenetics of a factor for multiploid sporocyte in barley; *Am. J. Bot.* **29** 451–456

Thompson M M 1962 Cytogenetics of *Rubus* III. Meiotic instability in some higher polyploids; *Am. J. Bot.* **49** 575–582

Vaarama A 1949 Spindle abnormalities and variation in Chromosome number in *Ribes nigrum*; *Hereditas* **35** 136–162

Venkateswarlu J and Krishna Rao M 1969 Chromosomal numerical mosaicism in some hybrids of the *Solanum nigrum* complex; *Genetica* **40** 400–406

Venkateswarlu J and Raja Rao K G 1979 Chromosome numerical mosaicism in a tetraploid interracial hybrid of *Physalis angulata* L.; *J. Cytol. Genet.* **14** 5–7

Yang S J 1964 Numerical Chromosome instability in *Nicotiana* hybrids I. Intraplant Variation among offspring of amphiploids; *Genetics* **50** 745–756

Yang S J 1965 Numerical chromosome instability in *Nicotiana* hybrids II. Intraplant Variation: *Can. J. Genet. Cytol.* **7** 112–119

Wilson G B 1950 Cytological effects of some antibiotics; *J. Hered.* **41** 227–231